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ANTIOXIDANT ACTIVITY AND TOTAL PHENOL CONTENT OF SOME SELECTED CEREALS

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ABSTRACT

Thirteen cereal varieties were evaluated for their DPPH radical, ABTS radical cation scavenging activity and free phenolic content. All investigated cereal exhibited significant antioxidant activities and contained significant levels of phenolic compounds. Based on the results obtained from this study, it is recommended whole grain products in the diet as a source of phenols and antioxidants.

INTRODUCTION

Cereals, such as barley, oats, rye and wheat contain minor components, which are assumed to have a vital role in the plant's defence system against biotic and abiotic stress. These compounds have antioxidative, antiinflammatoric and anticarcinogenic activities or have some other specific physiological activity (Pihlava et al, 2010). These compounds may combat oxidative stress in the human body by maintaining a balance between oxidants and antioxidants. This is particularly important because under severe oxidative stress excessive formation of reactive oxygen species (ROS) and free radicals can damage biomolecules, such as DNA, proteins, lipids and carbohydrates, and lead to numerous disease conditions (Halliwell, 1996).

Antioxidants are defined as molecules that, at low concentration and under specific assay conditions, can delay or prevent oxidation of an oxidizable substrate (Halliwell et al, 2004). Compounds with antioxidant capacity, and which are naturally present in food, are of great interest to the food industry and to consumers because of their potential value to prolong the shelf-life of foodstuffs by protecting them against oxidative deterioration and their possible beneficial effects on human and animal health (Zhou et al, 2004). A group of compounds that contributed to the antioxidant activity of cereal were considered phenolic compounds. (Zhao et al., 2008).

Phenolic compounds can be classified into a number of subgroups including phenolic acids, flavonoids, isoflavonoids, lignans, stilbenes, and complex phenolic polymers. Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerised compounds. Most naturally occurring phenolic compounds are present as conjugates with mono and polysaccharides, linked to one or more of the phenolic groups, and may also occur as functional derivatives such as esters and methyl esters (Bondia Pons et al, 2009).

In cereal grains, covalently bound phenolic acids are concentrated in the cell walls of the various grain tissues especially the aleurone and the pericarp-seed coat where they are esterified to the arabinose side groups of arabinoxylans.

Our previous studies showed that the ferullic acid was the predominant free phenolic compound present in the wheat grain, representing about 70% of the total free phenolic acids. Other phenolic acids in small quantities were p-coumaric, vannilic and syringic acids (Babeanu et al, 2010).

The objective of this study is to determine the antioxidant activity (DPPH radical scavenging activity and ABTS radical cation scavenging activity) and total phenolic content in selected cereals and identifying cereal variety with a good source of natural bioactive compounds.

MATERIAL AND METHOD

The cereals used in this study were produced at Research and Agricultural Development Station from Simnic. They are as follows: V1=triticale Haiduc; V2=triticale Titan; V3=triticale Gorun; V4=triticale Stil; V5=triticale Plai; V6=barley 6 r Cardinal; V7= barley 6 r Univers; V8=barley 6 r Dana; V9= barley 6 r Maresal, V10= barley 2 r Andreea; V11=oat Somesana; V12=sorghum NS5301; V13=Sorghum NS5302.

Extraction of samples: Extracts for the determination of phenols and antioxidant activity were prepared into 80% aqueous methanol (1:5 w/v) at 24°C for 16 h. The resulting slurries were centrifuged at 4000g for 5 min and the supernatants were collected.

Determination of total phenolic content (TPC): Each extract was mixed with Folin–Ciocalteu reagent and saturated sodium carbonate (Na_2CO_3) solution (Irmak et al., 2008). The mixture was allowed to stand at room temperature for 30 min and then the absorbance was recorded at 765 nm using a Thermo Scientific Evolution 600 UV-Vis spectrophotometer. The total phenolic content (TPC) was calculated using a standard curve prepared using gallic acid and expressed as µg of gallic acid equivalents (GAE) per gram.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is commonly used for the assessment of antioxidant activity. DPPH is a very stable organic free radical with deep violet color, which gives maximum absorption within the 515–528 nm range. Upon receiving a proton from any hydrogen donor, mainly from phenolics, it becomes yellow.

The capacity of cereal extracts to reduce the radical 2,2-diphenyl-1-picrylhydrazyl was assessed using the method of Kitts. A 0.075 mM (final concentration) DPPH solution in ethanol was mixed with wheat extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 20 min at 2 min intervals. The absorbance of the remaining DPPH radicals was measured at 519 nm. The normal color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. A blank reagent was used to study stability of $DPPH^{\cdot}$ over the test time. The scavenging activity of extracts was evaluated according to the formula: % scavenging = $[A_0-(A_1–A_S)]/ A_0]100$, where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract only. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of fresh whole grain. (µmol TE/g fw).

ABTS radical cation scavenging activity: The ABTS radical cation scavenging activity of the whole grain cereal extract was measured using the method of Zhao et al., (2008) with some modifications. ABTS was dissolved in water to a 7 mmol/l concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/l potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. 0.1 ml of the whole grain cereal extract was mixed with 2.9 ml of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of fresh material. (µmol TE/g fw).

RESULTS AND DISCUSSION

The results showed that free phenol content and antioxidant activity of cereals varied with studied genotypes (figure 1).

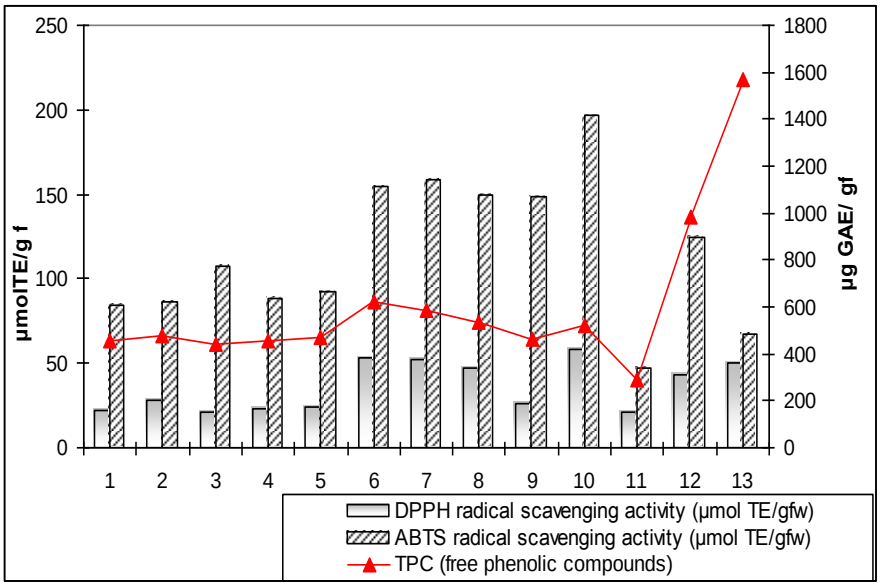

Figure 1. DPPH radical scavenging activities (µmol TE/g fw), ABTS radical cation scavenging activities (µmol TE/g fw) and free phenolic content (µg GAE/g fw) of different cereal varieties

The values of total phenolic content for barley ranged from 461to 620 µgGAE/g fw. These values are higher than the values determined for triticale (443-478 µgGAE/gfw) and for oat (287 µgGAE/gfw). The sorghum varieties had the highest phenolic content (983 and 1570 µgGAE/gfw).

All cereal varieties exhibited strong DPPH radical scavenging activity. The values of DPPH radical scavenging activity ranged from 21.08 to 53.26 µmol TE/gfw.

Cereals extracts were also measured and compared for their free radical scavenging activities against ABTS radical cation. Results are presented in fig 1. All cereals varieties showed significant ABTS radical cation scavenging activity. The values of ABTS radical cation scavenging activity for cereals samples ranged from 47 to 197 µmol TE/g fw. The results obtained by ABTS method differ from the results of the DPPH method.

Trolox equivalent values of cereal extracts obtained by ABTS assay were consistently higher than those obtained by DPPH assay. Thaipong K. et al (2006) and Zhao H et al (2008) also reported the same results when antioxidant activity was evaluated by both ABTS and DPPH assays. The different results from two methods might be due to different reaction kinetics between phenol and DPPH radical as well as ABTS radical cation over a similar range of concentrations (Campos et al. 1996). This was also probably due to various phenolic compounds present in the cereal extracts had different responses to various kinds of free radicals.

Positive correlations have been observed when DPPH radical scavenging activity and ABTS radical cation scavenging activity were compared with free phenolic compounds content, thus indicating that these compounds are responsible for the antioxidant activity.

CONCLUSIONS

Based on the results obtained from this study, it is recommended whole grain products in the diet as a source of phenols and antioxidants. Moreover, sorghum indicates the highest free phenol content and the barley varieties the highest antioxidant activity.

BIBLIOGRAPHY

Babeanu C., Trandafir I., Wheat grain's free phenolic acids profile RP-HPLC characterization, *Annals of the University of Craiova, The Chemistry Series*, XXXIX, No 2, 67-70, 2010

Bondia-Pons I., Aura A.M., Vuorela S., Kolehmainen M., Mykkanen H., Poutanen K., Rye phenolics in nutrition and health, *Journal of Cereal Science*, 49, 323-330, 2009

Campos, A. M., Lissi E. A., Kinetics of the reaction between 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) derived radical cation and phenols. International Journal of Chemical Kinetics, 29, 219–224, 1996

Halliwell, B., Antioxidants in Human Health and Disease, Annual Review of Nutrition, 16, 33-50, 1996

Halliwell B., Whiteman M., Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean., *British Journal of Pharmacology*, 142 (2), 231-255, 2004

Irmak S., Jonnala R., MacRitchie F., Effect of genetic variation on phenolic acid and policosanol contents of Pegaso wheat lines, *Journal of Cereal Science*, 48, 20-26, 2008

Kitts D. D., Wijewickreme A. N., Hu C., Antioxidant properties of a North American ginseng extract*, Molecular Cellular Biochemistry*, 203, 1-10, 2000

Pihlava J-M., Reinikainen P., Hietaniemi V., Kaukovirta-Norja A., The content of phenolics in barley, oats, rye and wheat cultivars, NJF Report, 6, No2, 2010

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D. H., Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis, 19, 669–675, 2006.

Zhao H., Fan W., Dong J., Lu J., Chen J., Shan L., Lin Y., Kong W., Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties., Food Chemistry 107, 296–304, 2008

Zhou, K., Laux, J. J., Yu, L., Comparison of Swiss red wheat grain and fractions for their antioxidant properties. Journal of Agricultural and Food Chemistry, 52, 1118–1123, 2004.